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Utilization of biomass waste empty fruit bunch fiber of palm oil for bioethanol production using pilot – scale unit

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Abstract

The major biomass byproduct from the palm oil industry is empty fruit bunches (EFBs). EFBs have a great potency as basic raw materials used for the fermentative production because they contain 37.3 – 46.5 % cellulose, 25.3 – 33.8 % hemicelluloses. Being abundant and outside the human food chain makes these cellulosic materials relatively inexpensive feedstocks for ethanol production and no conflict with the food supply. The bioconversion of EFBs to bioethanol consist of pretreatment for reducing the crystallinity of cellulosic material and removing of lignin, cellulosic hydrolysis or saccharification using a combination of enzymes for fermentable sugar production, and fermentation of produce fermentable sugars to bioethanol. In this study, pilot scale unit was set up for development and testing of a process for ethanol production based on enzymatic saccharification. Testing of the process including pretreatment of EFBs using alkali NaOH 10 %, saccharification using modified cellulase enzyme and fermentation of EFBs was carried out in the 350 L of fermentor tank using local strains *Saccharomyces cerevisiae* Mk, at 32°C for 48 h. The results of each process obtained are described.

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1. Introduction

Ethanol is a renewable energy source and can be produce domestically in response to days high-energy demand. Bioconversion of lignocellulosic waste materials to biofuel and chemical are receiving interest as they are low cost, renewable, and widespread in nature. The section headings are arranged by numbers, bold and 10 pt. Here follows further instructions for authors. Indonesia is the largest of palm oil producer in the world. Palm oil is among estate crops that play important roles in Indonesia's economy. It is one of the main sources of currency from non petroleum and gas sector besides rubber and cacao. Crude Palm Oil (CPO) is the main product of palm oil industries in the country. The process of fresh fruit bunches (FFBs) into CPO results in biomass wastes in large amount of empty fruit bunch fiber (EFBs). This EFBs, which is wastes in palm oil factories, is quite abundant, are often incinerated at the plant site which causes some air pollution. As a lignocellulosic biomass EFBs of palm oil contains cellulose, hemicellulose and lignin. Cellulose content of EFBs of palm oil is 37.3 – 46.5%, while hemicellulose and lignin content are 25.3 – 33.8% and 27.6 – 32.5%, respectively [1]. The types of main carbohydrates in EFBs of palm oil are glucan, xylan, and arabinan, each is 31.0; 17.3, and 0.5% [2]. Due to its high cellulose content, EFBs of palm oil is potential to be converted for the fermentative production of bioethanol, a promising alternative fuel to gasoline. The bioconversion of lignocellulosic waste of EFBs to bioethanol consist of pretreatment for reducing the crystallinity of cellulosic material and removing of lignin, cellulosic hydrolysis or saccharification using a combination of enzymes for fermentable sugar production, and fermentation of produce fermentable sugars to bioethanol. To fully utilize of EFBs as a feedstock for ethanol production, pretreatment is required to render the cellulose fiber more amenable to the action of hydrolytic enzymes. In this study, alkali pretreatment using NaOH solution were used to determined reducing of lignin content. In this study, pilot scale unit was set up for development and test of a process for ethanol production based on enzymatic saccharification. Testing of the process including pretreatment, saccharification and the fermentation step will be performed. The objective is to perform process integration, to provide basis data and demonstration of pilot plants for ethanol production from lignocellulosic materials. The focused study on the bioconversion of palm oil EFBs to ethanol particularly is on the analysis laboratory.

2. Materials and methods

2.1. Raw material

EFBs of palm oil fiber were collected from a Palm oil Plantation belongs to PT Perkebunan Nusantara VIII, in Pandeglang, Banten, Indonesia. The EFB was dried by sun shine and then chopped and hammer-milled to particle size 1-3 mm, then it was stored in sealed plastic bag at room temperature until use for pretreatment. Enzymes were provided by Novozyme, Korea. A cellulase complex and β -glucosidase were used to investigate enzymatic digestibility. The cellulase complex had an activity of 70 filter paper units (FPU) g⁻¹ cellulose. The β -glucosidase had an activity of 240 cellobiase units (CBU) g⁻¹. NaOH powder and others reagents used in this study were of analytical grade.

2.2. Equipment

Pilot plan equipment consist of two unit pretreatment, two unit saccharification tanks, four units fermentor tanks and one unit of distillation tank. For component analysis, supporting laboratory instrument is used such as Waters e2695 High Performance Liquid Chromatography with Refractive Index Detector to analyze the sugars and alcohol, and spectrophotometry to measure acid soluble lignin, and others chemical instrument to prepare all the analysis. 2.3

2.3. Pretreatment

Pilot scale was manufactured by Changhae Ethanol CO., LTD., Jeonju, Korea and designated as Changhae ethanol Multi Explosion (CHEMEX). One batch of the pretreatment was carried out using a 450 L mixer & exploder. NaOH solutions 10% w/w (250L) were used to pretreat 50 kg of ground EFBs with 10% of moisture content. Pretreatment were performed at temperatures 140-145°C and 4-7 kg/cm³ of pressure for 30 minutes. After treated of EFB were diluted with water in the 1st washing tank and insoluble solid were separated from aqueous solution by belt press filtration and transferred to the 2nd washing tank. EFB-treated were neutralized using water and H₂SO₄ 97% in the 2nd Washing Tank until pH 7-9. After washing and filtration the EFB-treated were transferred to buffer tank at temperature 80°C for sterilization, see Fig. 1.

2.4. Saccharification enzymatic

Saccharification enzymatic of EFBs was carried out in the 350 L saccharification tank. Two enzyme solutions, the cellulase complex and β -glucosidase were used for hydrolyzed pretreated of EFB. The EFB-treated from two batch process were transferred by screw conveyor from buffer tank to saccharification tank. Enzyme cellulase (Novozyme) 34 FPU (22 L) and enzyme β -glucosidase (Novozyme) 4.8 L was added simultaneously to saccharification tank. Saccharification process were performed at temperatures 50-52°C, pH 4.8-5.5 and mixed using agitator for 12 hours. The saccharification tank is equipped with a water jacket to maintain a constant temperature in the range of 50-52°C. Adjust pH 4.8-5.5 by using H₂SO₄ 97%. The vessel is also equipped with a pH-controlling device. The temperature, pH and agitation are monitored and controlled via a computer, using the software Cimon (KDT System Co. LTD, The Republic of Korea). After 12 hours of saccharification process, sampling for analysis of monosaccharide (glucose and xylose) content were withdrawn every 4 hours.

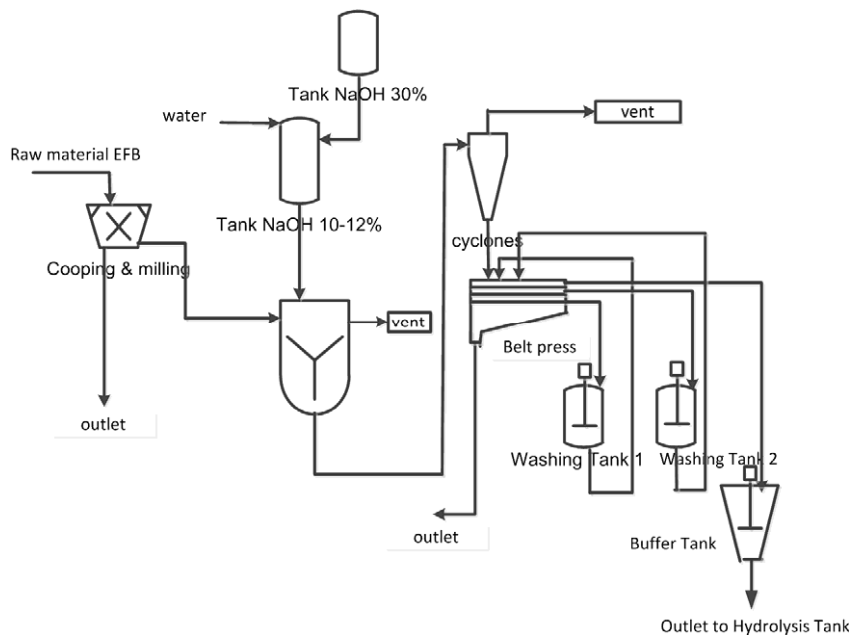


Fig. 1. Schematic flowsheet of pretreatment process

2.5. Fermentation

2.5.1. Inoculum preparation

The local strain of Indonesia *Saccharomyces cerevisiae* inoculum was prepared by growing the strain on solid YPD. The solid culture was incubated at 32°C for 48h. A single colony was transferred to a 50 mL erlenmeyer flask containing 10 mL of medium and grown at 32°C with agitation 120 rpm for 12h. This culture was used to inoculate the seed culture. The growth culture and preculture were performed in erlenmeyer flask (500 mL) containing 200 mL of medium. The medium containing per liter : 0.25% yeast extract; 0.25% peptone; 3.3% glucose; 0.03% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.10% KH_2PO_4 .

2.5.2. Fermentation process

The fermentation unit in the pilot plant consist of four tanks with volume capacity is 350 L for each tank. Fermentation of EFBs was carried out in fermentor No.3 containing 235 L product of saccharification, at 32°C and pH 6-5. The temperature, pH and agitation are monitored and controled via a computer, using the software Cimon (KDT System Co. LTD, The Republic of Korea). The hydrolysate was supplemented with 4 L yeast propagation of *Saccharomyces cerevisiae* Mk, Process of fermentation carried out for 36 hours. Sampling were withdrawn every 4 hr. The schematic flowsheet of Saccharification and Fermentation Process can be seen in Fig. 2.

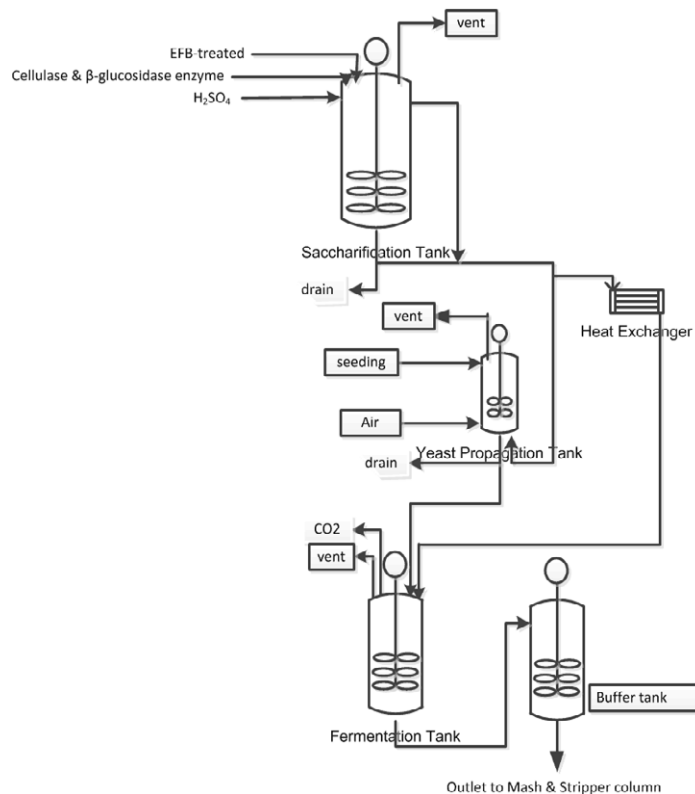


Fig. 2. Schematic flowsheet of saccharification and fermentation process

2.6. Analytical methods

The total solid, acid soluble lignin and acid insoluble lignin content of raw EFB and pretreated EFB were determined by using standard Biomass Analytical Procedures provided by National Renewable Energy Laboratory (NREL) [3]. The sugars content that are glucose and xylose in the hydrolysate, and ethanol concentrations were analyzed by using High Performance Liquid Chromatograph (HPLC). The HPLC (Waters, USA) system was equipped with AMINEX HPX 87H column, and a guard column, an automated sampler, a gradient pump, and a refractive index detector. The mobile phase was 5mM H₂SO₄ at flow rate of 0.6 mL min⁻¹ and oven temperature was maintained at 65°C. Prior to HPLC injection, all samples (derived from solids and hydrolysate) were neutralized with calcium carbonate, shake for 2 min, and filtered through 0.2µm syringe filters.

2.7. Yields

All yields are based on the theoretically available glucose content in the original raw material. The yield of ethanol is based on theoretically available amount of sugar in the raw material.

3. Results and discussion

3.1. Characteristic of EFB

The chemical composition of palm oil EFB used in this study in dry based is shown in Table 1. Based on HPLC carbohydrate analysis, the sugar fraction was 51.88%, the lignin fraction was 31.68% and ash fraction was 6.69% of the dry biomass.

3.2. Pretreatment

The chemical composition of pretreated raw EFB of palm oil was determined to get a figure about the result of pretreatment. By using the pretreatment process with NaOH as described before, the lignin and hemicellulose content in the EFB can be decreased to 11% and 5.69 % respectively, and the percentage of cellulose increased became 68.86% (Table 2).

Table 1. Chemical composition of palm oil EFB

No.	Component	Percentage (%)
1.	Lignin	31.68
2.	Cellulose ^(a)	37.26
3.	Hemicellulose ^(a)	14.62
4.	Extractive	1.34
5.	Ash	6.69

^(a) The percentage of cellulose and hemicellulose were calculated based on the concentration of glucose and xylose using an anhydro correction of 0.88 for C-5 sugars (xylose) and a correction of 0.9 for C-6 sugars (glucose) [4].

Table 2. Chemical composition of pretreated palm oil EFB*)

No.	Component	Percentage (%)
1.	Lignin	11.02
2.	Cellulose(a)	68.86
3.	Hemicellulose(a)	5.69

*) The data are the average of carbohydrate and lignin content from 2 batch pretreatment process

3.3. Ethanol production according to enzymatic hydrolysis and fermentation time

During the operation pilot scale process, all units performed well. NaOH solution was used for pretreatment, glucose was produced by enzymatic saccharification and fermentation using *S. cerevisiae*. The fermentation mash was analyzed by HPLC-RI to get the data of the remain sugars and concentration of ethanol produced for every 4 hours. In the chromatogram of fermentation mash, that can be seen in Fig.1, the peak of glucose, xylose and ethanol have retention time 9.4, 10.1 and 22.5 minute respectively as can be seen in Fig. 3.

Alkaline pretreatment of lignocelluloses with NaOH can remove or modify its lignin by fracturing the ester bonds that form cross-links between xylan and lignin, thereby increasing the porosity of biomass. Hemicellulose, which are branched and heterogeneous polymers of pentoses and hexoses, can relatively easily hydrolyzed to their monomers. On the other hand, cellulose is a crystalline polymer that cannot easily be hydrolyzed by chemicals [4]. Calculation based on the data showed that after pretreatment, washing and filtering, the amount of cellulose from the raw material from two batches pretreatment process (100 kg EFB) was 30.99 kg. The theoretical conversion of cellulose to glucose was 34.1 kg (kg of cellulose x 1.1). After 12 hours of saccharification process, the amount of glucose production was 20.92 kg. Therefore, the conversion of glucose from cellulose in saccharification process was 67.5%. Thereafter saccharification broth was transferred to the fermentation tank. pH condition and ethanol profile in the pilot fermentor No. 3 is figured in Fig. 4.

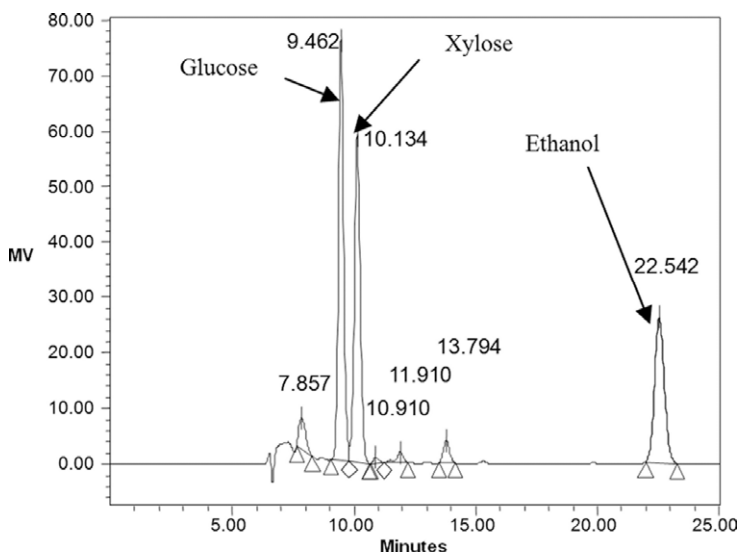


Fig. 3. Chromatogram of fermentation result

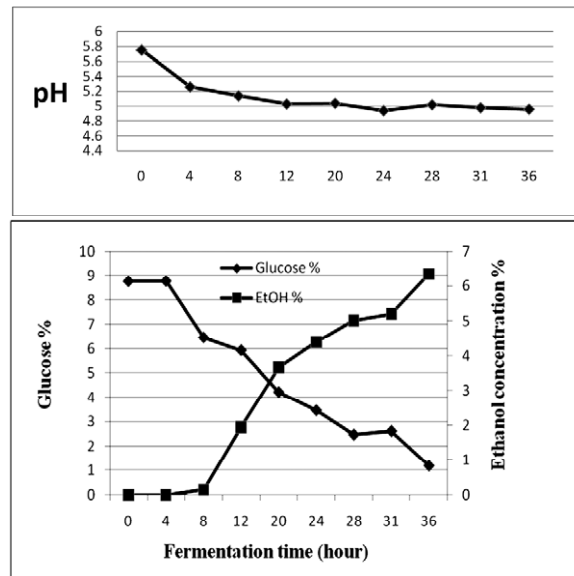


Fig. 4. Time course profile of ethanol production and glucose concentration carried out in pilot fermentor.

It can be seen in Fig 4. the profile of ethanol production in pilot fermentor No.3 with 239 L of working volume, fermentation by *S.cerevisiae* Mk. Accordance with enzymatic saccharification and fermentation time, decreasing sugar level and increasing ethanol production. In the case of enzymatic saccharification and fermentation were simultaneously performed, ethanol concentration reached 6.36% v/v during 36 hours fermentation which is close to 12.08 kg. Comparison to the theoretical ethanol production of available glucose in the raw material, the yield of ethanol production in fermentor No. 3, reached 69%. Xylose do not contribute to the yield since the fermenting organism *S. cerevisiae* does not ferment this sugars. However enhanced yields are expected.

4. Conclusion

This work took a major step toward addressing a compelling need for comparative data on the performance of leading the pilot scale unit to study the entire integrated process, the first evaluation of biomass pretreatment and subsequent enzymatic hydrolysis and fermentation for ethanol production. As expected the ethanol production process from palm oil EFB using NaOH pretreatment look effective, and it may be feasible for the high production of bioethanol of lignocellulosic waste in the near future.

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